PATENT
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IN THE U.S. PATENT AND TRADEMARK OFFICE

Applicant:	BRADY et al.	Conf.:	9468
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For: IDENTIFICATION OF GENES HAVING A ROLE IN THE PRESENTATION OF DIABETIC NEPHROPATHY

DECLARATION UNDER 37 C.F.R.§1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I Finian Martin, do hereby declare the following.

I am inventor of the claimed invention of the above-captioned application. I am also one skilled in the field of the invention and fully knowledgeable of the state of the art of the invention.

In my opinion, the gene represented by SEQ ID NO:1 is differentially up-regulated in response to glucose. In addition, the gene of SEQ ID NO:1 and the protein encoded from the gene are involved with the presentation of diabetic nephropathy.

I. Differential up-regulation of SEQ ID NO:1

The gene of SEQ ID NO:1 is also known as IHG-1. As shown in attached Figure 1, the gene of SEQ ID NO:1 is differentially up-regulated in response to glucose.

In the experiments of Figure 1, mesangial cells were exposed to 5mM glucose (control) or 30mM glucose for seven days. Quantitative real time PCR analysis of the mRNA of IHG-1 shows that the expression of IHG-1 (SEQ ID NO:1) in mesangial cells increases by a factor of 29 when cultured with 30 mM glucose, compared to the normal 5 mM glucose concentration. Thus, SEQ ID NO:1 is differentially expressed in the presence of glucose.

II. Involvement of SEQ ID NO:1 (IHG-1) in diabetic nephropathy

In my opinion, one skilled in the art would conclude that the gene of SEQ ID NO:1 and the protein encoded by the gene are involved in the presentation of diabetic nephropathy.

The protein encoded by SEQ ID NO:1 (IHG-1) shows a characteristic cellular distribution in mesangial cells and other mammalian cells. The protein encoded by SEQ ID NO:1 specifically associates with the mitochondria. See Figure 2 of the declaration.

In the experiments of Figure 2, primary human mesangial cells over-expressing V5-tagged IHG-1 (A) and mink lung cells

over-expressing V5-tagged IHG-1 (B) wer stained with fluorescent-conjugated antibodi s to determine IHG-1 localization. The immunochemistry studies of Figure 2 demonstrate the localization of IHG-1 to the mitochondria of the cells.

It is accepted in the art that a major molecular mechanism for diabetic nephropathy progression is the production of reactive oxygen species in mitochondria of mesangial cells. One skilled in the art would predict from the differential expression of SEQ ID NO:1 by glucose and the localization of the IHG-1 protein encoded by SEQ ID NO:1 that SEQ ID NO:1 is involved with the diabetic nephropathy.

I each hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

By _____ Date 04 March 2004

Attachments: Figures 1 and 2

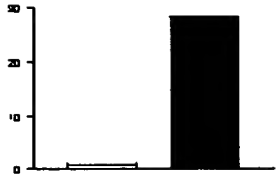

Figure 1 IHG-1 mRNA levels are increased by high extracellular glucose in human mesangial cells.

Influence of high ambient glucose on IHG-1 mRNA levels in human mesangial cells. Mesangial cells were exposed to 5 mM glucose (white box)) and 30 mM glucose (black box) for 7 days. Quantitative real time PCR analysis of IHG-1 expression, data are presented as ratio of target gene to ribosomal RNA control. IHG-1 levels are increased approximately 29-fold following exposure to 30mM glucose for 7 days.

A

B

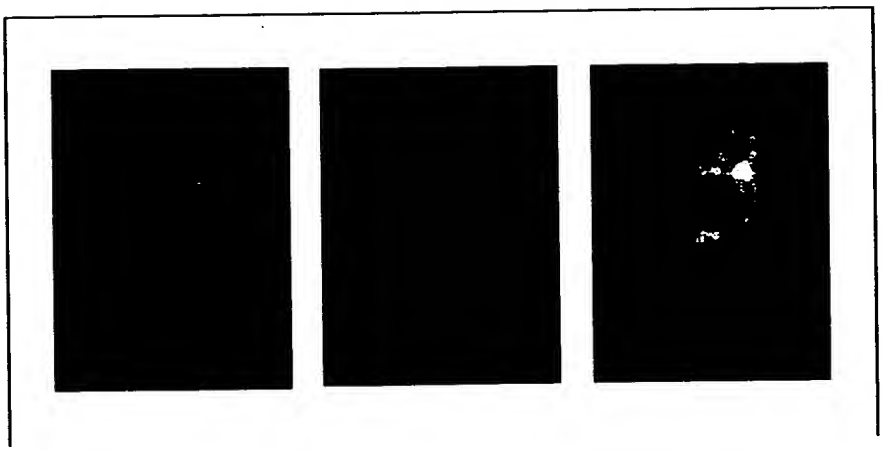

Figure 2: IHG-1 Expression is Localised to the Mitochondria

A. *Immunocytochemistry of Primary Human Mesangial Cells (HMCs) overexpressing V5 tagged IHG-1.* HMC were transiently transfected with V5-tagged IHG-1 in PCDNA6. Cells were fixed in 3.7% paraformaldehyde, permeablised and stained with anti-V5 and FITC conjugated secondary antibody.

B. *Immunocytochemistry of Mink Lung Cells overexpressing V5-Tagged IHG-1.* Cells were treated as in A and co-stained for the mitochondrial protein MnSOD and FITC conjugated secondary antibody (left panel) and anti-V5 and Texas Red conjugated secondary antibody (middle panel). The merged image (right panel) shows that IHG-1 is localised to the mitochondria.